Chapter One

History and Introduction of Bioreactors

WHAT IS BIOREACTOR

A bioreactor may refer to any manufactured or engineered device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel or glass.

A bioreactor may also refer to a device or system meant to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering or biochemical engineering.

On the basis of mode of operation, a bioreactor may be classified as batch, fed batch or continuous (*e.g.* a continuous stirred-tank reactor model). An example of a continuous bioreactor is the chemostat.

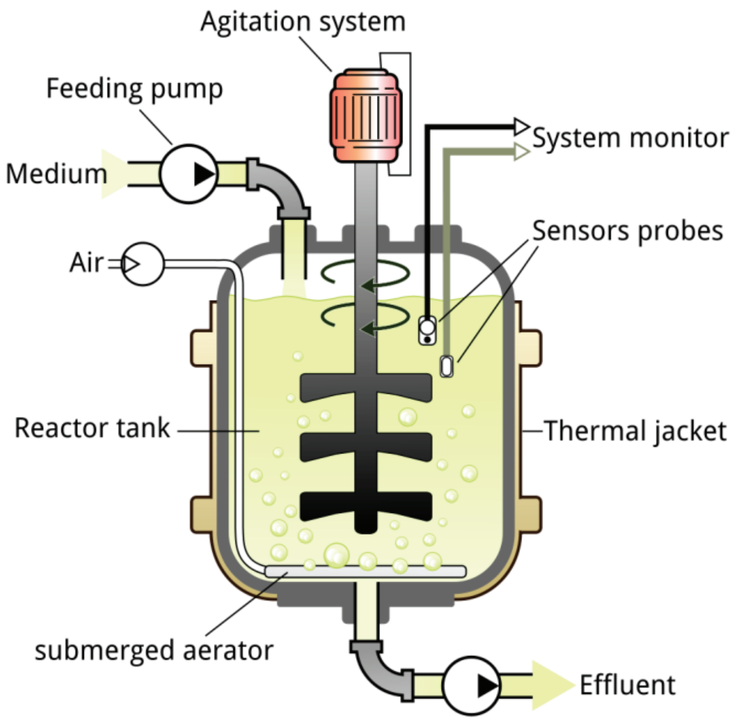

Fig. 1: Showing the Basic Bioreactor

History

Under this subhead we shall see the chronological developments of bioreactor.

1946

New Brunswick Tool & Die Company opens its doors as a 4-man shop in New Brunswick, N.J., USA, started by brothers David and Sigmund Freedman.

1949

Graduate students of Dr. Selman Waksman at Rutgers University attempt to isolate an antibiotic-producing bacterium for treating infection, but are stalled by frequent failure of their test-tube shaking apparatus. The Freedman brothers offer to build a better machine—the New Brunswick Shaker is born.

1952

Dr. Waksman is awarded the Nobel Prize in Medicine for isolating streptomycin—the first antibiotic successful in treating tuberculosis—creating

an instant demand for the New Brunswick Shaker. Over the next 25 years, NBS equipment goes on to be used in the discovery and production of virtually every antibiotic in commercial use.

1950 & 60s

Reciprocating shakers for more rigorously mixing cultures and chemicals; the first water bath shakers which provide temperature control for culturing micro-organisms; multi-tiered shakers for mass screening antibiotic compounds and producing vaccines; Psychrotherm—the first refrigerated incubator shaker; the Model G25 large-capacity console-style incubator shaker, and G76 water bath shaker—which unbelievably are still operating in labs around the world, some forty-plus years after they were first introduced. The world's first commercial fermenters—single vessel and multi-vessel systems, a 40-liter pilot plant fermenter and a continuous culture apparatus are introduced. Autoclaves for vessel sterilization, freeze dryers, colony counters and the first tissue culture roller drums are also developed.

1970s

New product "firsts" abound: NBS designs the world's first microbiological air samplers. Our slit-to-agar air samplers have been used in the Gulf War to aid in the detection of biological warfare, in Post Offices for the detection of anthrax, and in clean room environments to ensure the sterility of products during the manufacturing process. NBS custom-builds the world's first major "cell culture factory" for the University of Alabama, used to grow lymphoblastoid cells for the production of vaccines.

1980s and 1990s

NBS introduces CelliGen—a benchtop bioreactor specialized for animal cell culture and a pre-cursor to the CelliGen Plus. Over the years, enhancements such as a new, low-shear Cell Lift Impeller and packed-bed option enable researchers to dramatically increase product yields over conventional technologies.

NBS introduces the first automated bench top agar sterilizing system. The AgarMatic is a smaller version of our original floor model sterilizer, and when coupled with our new, automated PourMatic dish filler, provide hospitals and clinics with a labor-saving system, capable of sterilizing, pouring and stacking 320 plates in under an hour. NBS develops the world's first microprocessor-

controlled shakers. Innova® shakers mark a new era in the use of microchips to precisely control equipment setpoints, alarms, running time, speed and temperature. NBS has subsequently developed over a dozen new or replacement Innova shaker models, with ever-enhanced capabilities, continuing their role as our flagship products.

2000

Our first stackable shakers are introduced. Benchtop Innova 4200/4230 and large-capacity Innova 4400/4430 offer dual-temperature programming to automate switching between two temperatures on a programmed basis.

2001

We introduce the BioFlo 110, a benchtop fermenter/bioreactor with modular design allowing system expansion as needs or budgets grow. Capable of regulating up to four vessels simultaneously, the advanced controller enables users to view and change process parameters in English, French, German or Spanish.

2002

Researchers, working in conjunction with the World Health Organization, develop a new low-cost method of producing human rabies vaccine, helping to prevent a disease which kills 30,000 people each year. The protocol, which uses a 30-liter NBS bioreactor equipped with Cell Lift Impeller, turns out a million doses of vaccine and requires just three technicians to operate.

2003

NBS introduces the world's first modular, large-scale fermenters, cutting lead time for order to delivery from an industry-standard six or more months to an average of just 12 weeks. BioFlo Pro fermenters additionally make it easy to perform upgrades, at any time, pre- or post-delivery. For smaller scale needs, NBS introduces the full-featured BioFlo 410, a sterilizable-in-place benchtop fermenter/bioreactor with touchscreen interface, providing all the capabilities and features of a large-scale system in a benchtop unit.

2005

The Innova U360, a new slender upright ULT freezer is launched, designed to fit where other freezers cannot. It rounds out a line of 10 freezer models. NBS

introduces an inexpensive, disposable cell culture system known as FibraStage™. The labor-saving system uses just one to four disposable bottles, pre-filled with NBS' FibraCel® disks, to produce yields equivalent to dozens of spinners, or hundreds of T-Flasks or rollers. The BioFlo Pro line is expanded again, this time to include a series of modular, large-scale bioreactors for cell culture.

2006

NBS introduces two new CO_2 Incubators - Innova CO-170 and Excella CO-170, offering greater internal space without impacting footprint. Fourteen new shakers are introduced, including four new bench and floor model shakers in our top-of-the line Innova range; two new space-saving stackable I-26 & I26R incubator shakers; and an all new Excella™ line providing an economical alternative to Innova models The next-generation BioFlo 310 fermenter/ bioreactor is brought to market, capable of controlling up to four fermenters from an easy-to-use touchscreen controller. For the first time on the benchscale, the fermenter can integrate signals from up to ten external devices, including gas analyzers, sensors and scales, for optimized process control.

Fermenters and Bioreactors

In general terms, a fermenter is something that, as its name would suggest, ferments; this is, however, not a simple process. The process of fermentation has been known of for thousands of years, but has been mainly used over this time to the glucose found in various fruits, seeds and tubers into alcohol, later used for human consumption. In recent times, however, with increased knowledge of bacteria and fungi, fermenters have been put to a more (some would say) productive. A fermenter, then, is simply put an optimal environment for bacteria and/or fungi to grow in, and the cultivation of said organisms will yield a desirable substance use. A bioreactor is a vessel in which is carried out a chemical process which involves organisms or biochemically active substances derived from such organisms. Bioreactors are commonly cylindrical, ranging in size from some liter to cube meters, and are often made of stainless steel. in brief bioreactor can be considered as a large scale operation whose volume/ capacity ranges to several litres whereas fermenter only ranges ~2 litres. Bioreactor system used growth and is a for the maintenance of a population of mammalian or insect cells whereas Fermenter is a system used for the growth and maintenance of a population of bacterial or fungal cells. There is also geomerical difference between Fermenter and Bioreactor taller vessels are used

for bacterial processes to improve oxygen mass transfer whereas shorter vessels for mammalian cell culture to improve mixing.

There is also a difference in the aeration systems of the bioreactor and fermenter that is as follows:

Bioreactors

—Low gas flow rates typically on the order of 0.01 VVM.

—Inlet gas is a mixture of Air (for DO control and CO_2 stripping), Oxygen (for DO control without excessive gas flow rates), and CO_2 (for pH control).

—Foaming usually not a problem

Fermenters

—High gas flow rates, typically on the order of 1-1.5 VVM.

—Inlet gas is primarily air.

Occasional applications require oxygen enrichment.

—Foaming is frequently a problem.

—Oxygen transfer rate is usually the limitation

There is a difference in the agitator of the fermenter and the bioreactor also which is as follows:

Bioreactor Agitator

—Low shear

—High mixing capacity

—Power input typically

—1kw/1,000 liters

—Primary scaling criteria is mixing time

Fermenter Agitator

—High power input

—Radial impellers (Rushton turbines) are common – high speed

—Power input up to 10 kw/1,000 liters typical

—Primary scaling criteria is oxygen transfer rate

In this book we are definitely discussing about the bioreactors and not the fermenters, so we therefore turn our track towards bioreactors.

BIOREACTORS AND INTRODUCTION

The aerated bioreactor for solids processing is a 3-phase (solid–liquid–gas) multiphase system. The solids phase contains the adsorbed contaminants, the liquid phase (process water) provides the medium for microbial growth, and aeration complicates the system. Nutrients and adapted bio-mass may be added to enhance breakdown. Furthermore, process conditions (temperature, pH, O_2 level, etc.) can be monitored and to some extent controlled. Regarding the bioreactor configuration there are two major topics:

(1) Physical state of the multiphase system:

(I) bioreactors with a restricted solids hold-up: slurry reactors (typical solids hold up <40 wt%),

(II) bioreactor with restricted humidity: solid state fermentation (solids content >50 wt%).

(2) Operation mode:

(I) batch operation; no fresh material is introduced to the bioreactor during processing, the composition of the content changes continuously;

(II) continuous operation (plug flow); fresh material is introduced and treated material removed during processing, the composition in the reactor remains unchanged with time (Levenspiel, 1972); in practice semi-continuous operation is often used (interval-wise feeding and removal giving small fluctuations in the reactor).

Bioreactors are used for carrying out biochemical processes which employ microbes, fungus, plant cells or mammalian cell systems for production of biological products. The bioreactors provide a controlled environment for the production of metabolites which can help to achieve the optimal growth of microbes.

Why Use a Reactor?

Reactors permit the controlled development of a biofilm so that in replicate trials, rather consistent biofilm samples are produced to that the measurements of such parameters as biofilm mass, thickness, metabolic activity, cell number and resistance to chemical or physical challenge can be made.

Reactor systems can be established that permit investigators (that's you) to control many experimental variables including:

- Chemical variables
  - o Substrate type (sugars, proteins, lipids, etc.)
  - o Substrate (nutrient) concentration
  - o pH
  - o Inorganic components (nitrogen and sulfur source etc.)
  - o Dissolved Oxygen
  - o Growth Inhibitors (antimetabolites, disinfectants)
- Physical variables
  - o Temperature
  - o Fluid shear stress
  - o Surface composition and texture (hydrophobic, hydrophilic, rough, smooth)
  - o Hydraulic residence time
- Biological
  - o Organism type (pure or mixed culture)
  - o Organism concentration

What Sorts of Reactors are There?

In the early days of biofilm research, commercial reactors were available from a limited number of suppliers and those that were available were very expensive. Investigators like Bill Characklis, Barry Pyle and Gordon McFeters improvised using Mason jars ® and other readily available materials as reactor vessels. Today, commercial reactor types are readily available but they are still very expensive.

In this collection we will revert to the fields infancy and provide instructions for building inexpensive but serviceable reactors from readily available materials, many of which you have in the lab already.

Bioreactors have also been used for many years in areas in different fields of biotechnology. They have been used in diverse areas such as in fermentation, in

Fig. 2: An early batch reactor, circa. 1992 constructed from a Mason Jar.

water treatment, in food processing and in the production of pharmaceuticals. All of these bioreactors are devices in which biological or biochemical processes develop under a closely monitored and tightly controlled environment. Bioreactors have been used in animal cell culture since the 1980s in order to produce vaccines and other drugs and to culture large cell populations. Bioreactors for use in tissue engineering have progressed from such devices.